COLORADO UNIV AT BOULDER DEPT OF AEROSPACE ENGINEERI--ETC F/6 6/18 MICROWAVE EFFECTS ON LEARNING AND MEMORY IN MICE.(U) SEP 80 M W LUTTGES AFOSR-80-0036 AD-A094 788 AFOSR-TR-81-0091 UNCLASSIFIED l or l

UNCLASSIFIED	
SECURITY CLASSIFICATION OF THIS PAGE (When Date Entered)	READ INSTRUCTIONS
JA REPORT DOCUMENTATION PAGE	PASTOVE COMPLETING FORM
AFOSR/TR-81-0091 AD-A094	788
MICROWAVE EFFECTS ON LEARNING AND MEMORY	FINAL
7. AUTHOR(s)	6. PERFORMING ORG. REPORT NUMBER 8. CONTRACT OR GRANT NUMBER(5)
M. W. Luttges	AFOSR-8Ø-Ø036
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT, PROJECT, TAS AREA & WORK UNIT NUMBERS
University of Colorado Department of Aerospace Engineering Science	1 61102F 2312 A5 (17) A 5
11. CONTROLLING OFFICE NAME AND ADDRESS Air Force Office of Scientific Research (NU)	/ 30 Sep 80 /
Bolling AFB DC 20332	13 NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Offic	e) 15. SECURITY CLASS. (of this report)
	Unclassified
1 1 2 1 2 5	15a. DECLASSIFICATION DOWNGRADIN
	1 138, DECENSIFICATION DOWNGRADIN
Approved for public release; distribution unlimit	SCHEDULE
	ted.
Approved for public release; distribution unlimi	ted.
Approved for public release; distribution unlimi	ted.
Approved for public release; distribution unliming the state of the abstract entered in Block 20, if different the state of the abstract entered in Block 20, if different the state of the abstract entered in Block 20, if different the state of the abstract entered in Block 20, if different the state of	ted.
Approved for public release; distribution unliming the state of the abstract entered in Block 20, if different the state of the abstract entered in Block 20, if different the state of the abstract entered in Block 20, if different the state of the abstract entered in Block 20, if different the state of	ted.
Approved for public release; distribution unliming a provided for public release; dis	ted.
Approved for public release; distribution unliming a provided for public release; dis	ted.
Approved for public release; distribution unliming a supplementary notes 18. Supplementary notes 19. KEY WORDS (Continue on reverse side if necessary and identify by block num	ted. ted. ted. ted.
Approved for public release; distribution unliming a provided for public release; dis	ted. ted. ted. ted.
Approved for public release; distribution unliming a supplementary notes 18. Supplementary notes 19. KEY WORDS (Continue on reverse side if necessary and identify by block num	ted. (from Report) (ber)
Approved for public release; distribution unlimit 17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different 18. SUPPLEMENTARY NOTES 19. KEY WORDS (Continue on reverse side if necessary and identify by block num 20. ABSTRACT The effects of microwave irradiation to	ited. (from Report) (steer) (steer)
Approved for public release; distribution unlimit 17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different 18. SUPPLEMENTARY NOTES 19. KEY WORDS (Continue on reverse side if necessary and identify by block num 20. ABSTRACT The effects of microwave irradiation we memory in mice. Also, preliminary studies were of	ited. (from Report) (steer) (steer)
Approved for public release; distribution unlimit 17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different 18. SUPPLEMENTARY NOTES 19. KEY WORDS (Continue on reverse side if necessary and identify by block num 20. ABSTRACT The effects of microwave irradiation was memory in mice. Also, preliminary studies were of underlying physiological mechanisms related to	vere assessed on learning and completed to begin an assessment the observed microwave effects
Approved for public release; distribution unlimit 17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different 18. SUPPLEMENTARY NOTES 19. KEY WORDS (Continue on reverse side if necessary and identify by block num 20. ABSTRACT The effects of microwave irradiation we memory in mice. Also, preliminary studies were of underlying physiological mechanisms related to on behavior.	vere assessed on learning and completed to begin an assessment the observed microwave effective or the observed microwave effective or the observed microwave expositions.

average power levels, small but reliable increases in performance were documented. All treatments were delivered posttrial for a 15 min. period. Sham-treated mice were treated in the same manner as exposed mice except that no radiation was delivered. Repeated replications with different aged animals produced the same effects. The microwave facilitation was observed in both automated active avoidance testing and in single-trial, passive avoidance testing. The modest facilitation effect was observed when the mice were tested at 20 days after original training.

Using a different irradiation chamber, the studies were repeated. The new chamber used an impedance matched horn in which the mice were restrained in a constant orientation relative to the microwave fields. In this test configuration the mice received an average power level of 22mW/cm². Once again, small but reliable amounts of performance facilitation were observed. The new, average power levels were associated with irradiation-induced, rectal temperatures which were higher in irradiated than sham-treated mice. As observed earlier, training-induced temperature increments dissipated more quickly in control than exposed mice during the posttrial treatment period.

To obtain independent indices of the microwave effects in nonbehavioral measures, a method was devised to evaluate alterations in blood brain barrier and polypeptide synthesis activities. Preliminary observations appear to corroborate the microwave effects on 35 S-methionine uptake and incorporation into brain proteins.

Additional studies should explore both the limits of the unexpected behavioral alterations and should focus on the underlying physiological mechanisms which support such alterations.

Accessi	on For		
NTIS G	RA&I B msed]]
Justiff	cation	n	
By	bution abilit Avail Spec	ty Cod and/o	es
+11	-	+	

AFOSR-TR- 81-0091

FINAL AFOSR-80-0036

Microwave Effects on Learning and Memory in Mice

Abstract. The effects of microwave irradiation were assessed on learning and memory in mice. Also, preliminary studies were completed to begin an assessment of underlying physiological mechanisms related to the observed microwave effects on behavior.

Using a resonant microwave irradiation chamber in which mice were exposed following daily training to 3GHz pulsed microwave at approximately 18mW/cm^2 average power levels, small but reliable increases in performance were documented. All treatments were delivered posttrial for a 15 min. period. Sham-treated mice were treated in the same manner as exposed mice except that no radiation was delivered. Repeated replications with different aged animals produced the same effects. The microwave facilitation was observed in both automated active avoidance testing and in single-trial, passive avoidance testing. The modest facilitation effect was observed when the mice were tested at 20 days after original training.

Using a different irradiation chamber, the studies were repeated. The new chamber used an impedance matched horn in which the mice were restrained in a constant orientation relative to the microwave fields. In this test configuration the mice received an average power level of 22mW/cm^2 . Once again, small but reliable amounts of performance facilitation were observed. The new, average power levels were associated with irradiation-induced, rectal temperatures which were higher in irradiated than sham-treated mice. As observed earlier, training-induced temperature increments dissipated more quickly in control than exposed mice during the posttrial treatment period.

To obtain independent indices of the microwave effects in nonbehavioral measures, a method was devised to evaluate alterations in blood brain barrier and polypeptide synthesis activities. Preliminary observations appear to corroborate the microwave effects on 35 S-methionine uptake and incorporation into brain proteins.

Additional studies should explore both the limits of the unexpected behavioral alterations and should focus on the underlying physiological mechanisms which support such alterations.

The state of the state of state of REGEARCH (AFSC) and the state of th

81209056

Before attempting to test a large number of mice, several pilot tests were completed. Test parameters were set to achieve efficient learning without using excessive levels of footshock which might interact with the subsequent irradiation. Also, several animal restraint devices were tested to assure minimal microwave effects and to assure minimal interference with animal comfort. Finally, ten animals were irradiated and 10, sham-irradiated to assess rectal temperature alterations. These pilot studies were extended to cover temperature alterations induced by avoidance training and to characterize the effects of microwave exposure on temperatures previously elevated by training. Once these studies were complete we initiated formal, repetitive testing. To assess learning rates we characterized daily avoidance improvements across a five day training period and to assess long-term memory we retested all mice approximately 20 days after the last training day.

Methods

Animals. Approximately 100 HS mice, males and females, were used in these studies. Three separate repetitions of the study were completed using matched test groups which differed only in age from one experiment to the next. All animals were weighed prior to "exposed" or "control" group assignment. Groups were matched by weight and sex. The test animals were selected from group cages of ten mice each prior to subdividing according to test group assignments. Throughout the course of testing, animals were housed two per cage (one each; exposed, control) with free access to food and water while in this home cage.

Apparatus. Behavioral testing occurred in an automated, active-avoidance apparatus. To avoid mild footshock, animals must learn to turn a small wheel

apparatus. To avoid mild footshock, animals must learn to turn a small wheel mounted at the front of the test cage when a sound occurs. Failure to turn the wheel results in footshock delivered five secs after the sound is presented. Successful avoidance in the period between sound delivery and shock delivery terminates the sound, deactivates the shock circuit and records a "correct" trial automatically. At all times the total amount of wheel-turning is recorded as a measure of overall activity. Four test cages may be used simultaneously. The apparatus has been described in detail elsewhere (Andry and Luttges, 1973).

The microwave exposure was delivered from a B.J.Electronics Mdl. 80 power signal generator. The nominal output frequency was 3GHz pulsed in 5 usec epochs at 2500 Hz. The output was monitored by Polaroid Mdl. DU2 spectrum analysor throughout each irradiation treatment. The irradiation chamber was

constructed from a 1 cm thick wall, aluminum tube machined to incorporate the following features: (1) a 40 cm length varied to shorter lengths by a metal plunger in the end distal to the antenna, (2) a 10 cm (i.d.) circumference with small (1/8") airholes along the length as well as a half radius, hinged metal door, and (3) a half radius end reflector driven by synchronous motor at 2 rpm. The metal plunger was moved to achieve desired average power levels at the door used for positioning the experimental animals. A second tube with identical internal features was used for sham treatments.

The animals were placed in either tube by first restraining them in a plexiglas chamber ($10 \times 4.3 \times 3.1$ cm; length, height and width, respectively) which had end shapes with the same radius as the tube. Accordingly, the animals were positioned in the middle of the tube at the desired position. Prior periods of restraint adaptation resulted in relatively calm animals during the period of irradiation or sham treatment.

Rectal temperatures were measured with a thermister attached to a narrow-range, digital meter. Temperature equilibration time was less than five secs.

Procedure. Animals were separated into test caging as matched experimental and control pairs. At the same time tails were marked to preserve individual identity. Following two days of adaptation, testing was begun.

During testing the mice were placed into individual chambers and given 50 daily trials. The sound-shock (CS-UCS) interval was 5 secs and the average time between trials was 20 secs. Each trial lasted for 15 secs if the mice failed to turn the wheel earlier. The whole training period usually lasted for 30-40 min. Immediately after daily training, the animals were removed to the irradiation or sham-irradiation test chambers, respectively. Rectal temperatures were often obtained at this time as well as before training and after treatment. Both control and experimental animals were exposed to similar equipment noises and other stimuli. Irradiation levels were measured by a Simpson Microwave Power Meter (Mdl. 380) before and after treatment. Also, the levels were monitored by spectrum analysor during irradiation. After 15 mins (in most cases) of treatment both animals were removed and returned to home cages. The behavioral data were recorded and the counters were reset for the next tests. These procedures were repeated after daily tests but not after the 20 day retention test.

Results

Each of the three repetitions of this experiment revealed the same findings. As may be seen in Fig. 1, both control and exposed mice show significant active avoidance improvements across test days (p < 0.01, two-tailed). When these data were normalized and combined with those of the other two repetitions (N = 20 each, for control and experimental groups), the experimental mice exhibited significantly better (p < 0.05, two-tailed) avoidance learning than control mice. After more than 20 days without additional tests, all mice exhibit excellent retention. Although some mice perform slightly less well than they had earlier, there is no significant (p > 0.28; two-tailed) change in performance.

Since it was possible that the irradiated animals were more active than control animals and, thus, achieved higher apparent avoidance scores, the activity of the mice was analysed. The square root transformation plotted in Fig. 2 shows alterations in total wheel-turn activity across training. The microwave irradiated mice exhibit approximately 15 percent more wheel-turn activity than control mice. This difference is significant (p < 0.001, two-tailed). This observation does not indicate, necessarily, a direct microwave increment in overall activity. Higher numbers of successful avoidances may produce such a correlated increase in wheel-turn activity. In fact, wheel-turn activity divided by the number of successful avoidances indicates that experimental and control animals, alike, show more avoidances to correlate with more wheel-turns. As training progresses, wheel-turn activity decreases but successful avoidances continue to increase.

Passive Avoidance Studies

The active avoidance studies did not provide an unequivocal answer regarding the potential facilitative effects of posttrial irradiation. The microwave exposure increased activity and such increases could have been advantageous to animals in successful active avoidance. These effects would be subject to a different interpretation than effects related to more direct microwave alterations in learning.

To separate the possible activity effects from learning effects, we employed a task which penalizes animals for exhibiting increases in activity. In particular, a passive avoidance task requires an animal to remain inactive, withholding what might be considered a normal response. If microwave exposure increases activity, microwave-treated mice should do poorly on passive avoidance learning when compared to sham-treated, non-irradiated mice.

Methods

<u>Animals</u>. Fifty-two HS mice were used in these experiments. Half of the mice were assigned to sham-irradiated and half, to irradiated test groups. The mice were housed as described above.

Apparatus. Behavioral testing was conducted in a modified Jarvik box. An animal is placed in a small, bright starting box and then a door is lifted to provide access to an adjacent darkened box. The usual response is for the animal to retreat quickly to the darkened box. However, the grids of the floor of the darkened box are activated upon animal entry. The animals leave the electrified box quite quickly. When tested a second time the animals are extremely reluctant to enter the darkened box. Response performance is characterized by recording response latencies. Since many animals simply do not enter the darkened box a second time, a 300 secs maximal response latency cut-off is used. After this time has elapsed, the mice are removed from the test apparatus and the trial is terminated. The aversive footshock is approximately 2 ma for these tests.

Not only does this task differ from the previous one in the effects activity increases are likely to produce; but also, in the temporal alterations allowed in the experimental paradigm. In the latter case, the trained mice can be removed from testing and be subjected to microwave exposure within two-three minutes after a trial had been initiated. The previous experiment relied upon long periods of training. Thus, the microwave exposure was delivered long after the initiation of active avoidance training.

The microwave exposure and sham-exposure conditions were the same as described above.

<u>Procedure</u>. All mice were trained on the first experimental test day and retested 24 hours later. Shock was administered on the 24 hr test and all mice were tested again seven days later. As a measure of emotionality, bolus counts were completed after each test. The apparatus was cleaned thoroughly after each test to prevent gustatory cues from producing interactions of performance from one animal to the next.

Results

All mice, on the average, step from the lighted to the darkened box within one minute. At the 24 hr test many mice exhibit criterion response latencies of 300 secs. When tested seven days later all mice, experimental and control, exhibited criterion response latencies. Whereas very few boluses

are produced during training, many boluses are produced during the 24 hr test. These results are summarized in Fig. 3.

Because of response variance the exposed and sham-irradiated mice did not differ significantly (p < 0.08, two-tailed) on initial response latencies. It is clear, however, that control mice approach significantly longer initial response latencies. On the 24 hr test the two groups do differ significantly (p < 0.0001, two-tailed). The experimental group shows a larger number of criterion latencies than the control group. Also, the mean and median latencies are longer than those of control mice. Of the 26 mice in each group, more than half exhibited criterion performance. This asymptotic or "ceiling" effect may have limited the magnitude of differences between groups.

An analysis of bolus production revealed significantly (p < 0.05, two-tailed) more boluses produced by experimental as compared to control animals. Unfortunately, experimental and control mice differed on the initial trials as well. The control mice produced significantly more boluses (p < 0.003) than experimental mice during learning. Thus, these groups exchanged relative amounts of bolus production as a consequence of training.

Conclusions

The active and passive avoidance studies, together, show that microwave exposure facilitates learning. If activity were the main consequence of irradiation, passive avoidance performance would have been disrupted. Clearly, such disruption was not in evidence. The irradiated mice performed passive avoidance slightly, but significantly better than control mice.

It might have been tempting to relate bolus production to increased emotionality in irradiated mice. Although this cannot be disproved directly, it should be noted that naive, training trial mice produce few boluses. It does not seem unreasonable that the more well trained, experimental mice would produce more boluses simply because they exhibit better learning and retention.

Active Avoidance Study (18mW/cm²)

In order to characterize the temporal relations of microwave exposure to learning trials in regard to the effects produced, pretrial irradiation was attempted. These studies were conducted using the same methodologies as described earlier. The only procedural difference involved irradiation or sham-irradiation 15 min prior to behavior testing. Pre-trial irradiation was not done prior to the 20 day retesting.

Results

The irradiation and sham-irradiation treatments administered for 15 mins prior to daily 50 trial sessions seemed to produce somewhat less effective learning than observed in previous experiments. Rapid improvements in avoidance performance over the first three days were followed by low asymptotic curves over test days four, five and twenty.

Across the whole test schedule, the exposed mice exhibit more avoidances than the sham-exposed mice. This difference is significant (p < 0.001, two-tailed) even with the small number (eight per group) of animals tested. Similarly, the experimental mice show significantly (p < 0.01, two-tailed) more wheel-turn activity than the control mice.

Conclusions

Microwave irradiation produces avoidance enhancement whether delivered prior to or after training. The effect is seen with both active and passive avoidance. None of these effects can be related to direct associations of microwave exposure to the training situation since microwave irradiation is not present during actual training.

Active Avoidance Study (22mW/cm²)

In view of the preceding results we were concerned that our observations may have been unique to our irradiation procedures. Also, we became concerned that the analysis of actual microwave fields impacting the organism might be quite complex. Accordingly, we altered the irradiation procedure to a transmissive exposure scheme. An impedance matched horn was inserted into an exposure chamber lined with custom-made absorptive blocks. The resulting field strength was characterized and the absorption coefficients measured. Since this new exposure chamber was to be used, we also collected additional rectal temperature data. The modified exposure system is shown in Fig. 4.

Methods

All animal, apparatus and procedure details are similar to those noted above. These experimental animals and controls were treated appropriately posttrial. Treatments lasted 15 mins.

Results

Across all test days the experimental, irradiated mice exhibited post-trial, pretreatment temperatures which did not differ from control mice temperatures. After irradiation or sham-irradiation the two groups differed significantly (p < 0.05, two-tailed) in rectal temperature.

All mice $38.5^{\circ}C$ Exposed, posttreatment $38.9^{\circ}C$ Pretreatment Sham, posttreatment $38.4^{\circ}C$

The exposed and control mice did not differ significantly in avoidance learning, though the exposed mice exhibited higher average avoidance scores. The total wheel-turn activity for the two groups did differ significantly (p < 0.05, two-tailed) with the experimental mice exhibiting the largest number of wheel-turns. These results were quite reminescent of our earlier results using the resonant chambers and slightly less average power levels.

From these observations we conclude that the effect we have observed is unlikely to be related to specific field orientations relative to the exposed mice. Nevertheless, a more comprehensive study was designed to yield somewhat more clear findings. The major alteration was to be the use of extensive adaptation of the mice to the new restraining devices. Active Avoidance Study $(22mW/cm^2)$

Prior to testing all mice were given three days (15 min each) of adaptation to the restraining devices. Using the standard posttrial paradigm, all mice received five successive days of 50 trials each followed 20 days later by retesting. Ten mice were used in each group.

Results

The two groups did not differ in rectal temperatures measured immediately after training. And, both groups exhibited significantly lower body temperatures 15 mins later, after irradiation or sham-irradiation. As seen in Fig. 5, the experimental mice, due to microwave exposure, remained significantly (p < 0.01, two-tailed) warmer than sham-irradiated mice. Although these temperature changes are modest (< 1° C) they are quite reproducible.

The estimates of core temperature changes are associated with modest average differences in avoidance performance. Avoidance numbers do not differ between experimental and control groups. However, when avoidance successes are compared to number of wheel-turns, it is clear that the irradiated mice exhibit more response effectiveness than control mice (Fig. 6). These differences are significant (p < 0.01, two-tailed) and persist through the 20 day retention tests. These differences arise from the fact that exposed animals quickly adapt to the test situation and engage in wheel turning only during the CS-UCS interval. Unlike previous groups, the microwave mice seem less active than their respective controls in the test apparatus.

Active Avoidance Study (22mW/cm²)

Since the above studies indicate that microwave exposure facilitates avoidance learning whether administered prior to or following training, the temporal contiguity of treatment should be investigated. First, it seems possible that extended treatment durations should be evaluated. Secondly, the treatments may be moved systematically away in time from the daily training trials. Whereas the former strategy addresses perservering effects of longer duration exposures, the latter addresses proximity to sensitive physiological processes associated with training.

The following study is a first step in the direction of exposure duration. Using the same task as described earlier, we have simply doubled the posttrial exposure duration.

Results

Summation of avoidances are presented in Fig. 7. The control mice begin with performance inferior to that of irradiated mice. However, by the fourth training day the irradiated mice cease to exhibit improvement. In contrast, the control mice show continued benefit from the additional training. When the avoidance performance is subdivided into early (days 1-3) and late (days 4-5, 20) periods, the irradiated mice perform better than control mice (p < 0.05, two-tailed) in early trials but more poorly than controls (p < 0.04, two-tailed) on later trials.

Indices of wheel-turns and response efficiency show the same types of alterations. Also, these indices indicate no remarkable alterations which may indirectly account for the variations in avoidance scores between groups.

Conclusions

The behavioral studies, together, show the following effects of microwave irradiation:

- (1) Active avoidance learning and memory are facilitated.
- (2) Passive avoidance learning and memory are facilitated.
- (3) Activity and emotionality scores are enhanced.
- (4) Microwave effects are produced even when microwave is not present during training.
- (5) Microwave effects are produced whether exposure is given prior to or following training.
- (6) Microwave exposure is observable as a change in thermal load imposed on the mice. This thermal load is smaller than that produced by active avoidance training.

- (7) Random incidence and ordered field exposures result in similar effects on avoidance learning.
- (8) Longer periods of exposure appear to disrupt learning and memory when such exposure is imposed repeatedly over successive days.

 Autoradiographic Studies: Uptake and Incorporation (22mW/cm²)

All of the behavioral alterations suggest a reliable consequence of microwave irradiation on some underlying physiological mechanism supportive of improved avoidance learning and memory. Taken to longer periods of irradiation, the same mechanism may result in detrimental effects on learning and memory. At least one postulated effect of microwave irradiation, altered blood-brain barrier permeability, could relate to some of the behavioral effects we observed. This possibility has been the subject of several preliminary autoradiographic studies in our laboratory. Even if specific information on blood-brain barrier activity is not readily obtained, we have initiated autoradiographic studies since such work is likely to reveal any overall changes in brain permeability and activity.

Methods

<u>Animals</u>. The HS mice were similar to those used in behavioral studies. They were maintained as paired control and experimental subjects in small home cages. They had free access to food and water in the home cages.

Apparatus. Microwave exposure apparatus was the horn system described earlier. All mice to date have received 15 mins of exposure $(22mW/cm^2)$ or sham-exposure.

The animals were injected using a 32 gauge cannula attached to a 50 μ 2 Hamilton microsyringe. A perfusion set-up allowed subsequent saline and formalin washes of the brain tissues prior to removal from the mice. The brain sections were prepared using a rotary A-O microtome with a freeze-stage attachment. Dried sections were coated with nuclear tract emulsion (Kodak NTB2) and subsequently developed with Kodak D76 developer. Photographic prints were made from each section directly by using such sections as a photographic negative. More detailed photographs were prepared by photomicroscopy using either an A-O or Nikon microscope with planar objectives.

<u>Procedure.</u> Trained mice or naive mice were exposed to the usual microwave irradiation or sham-irradiation followed by rapidly-induced chloroform narcosis. At the depth of anesthesia, each mouse was injected with $25\mu\text{Ci}$ of $^{35}\text{S-methionine}$ in $25\mu\ell$ of saline. The injections were made beneath the occipital plate directly into the cisternae magnum. The mice recovered righting reflexes

within five mins after injections. Any mouse exhibiting abnormal locomotion was discarded from the study. Pulse labeling with the 35 S-methionine was allowed for 15 min. At this time, the mice were sacrificed by cervical dislocation and perfusion was initiated through the heart. Saline was used to clear blood from the system and formalin (10%) was used to fix the brain in situ. The brain was then removed to 20 volumes formalin (10%) for two days. The tissue was then brought to a 50%(v/v) concentration of ethanol and used for the preparation of frozen sections. Sections cut at 40μ thickness were dried on slides and coated with a thin layer of prewarmed, liquid emulsion. A dozen slides with three sections each were coated with emulsion for each animal. Three days later the first slides were developed with D-76. The remaining slides were developed over periods up to three weeks depending upon the amount of radiographic exposure brained. All slides were counterstained with eosin.

Permanent documentation was achieved with photographs prepared directly from the slides as negatives or from photomicrographs. Quantifications arise both from exposure densities at time of development and from patterns of labeling. Detailed comparisons arise from coronal sections prepared from normal animals and stained by hematoxylin-eosin procedures.

Results

Autoradiographic data obtained to date indicate two major findings related to microwave exposures:

- (1) Microwave irradiation results in more ³⁵S-methionine uptake and incorporation than observed in sham-irradiated mouse brains.
- (2) Microwave irradiation results in preferential labeling of limbic system structures as compared to sham-irradiated labeling.

Sample coronal autoradiographs are shown in Fig. 9. The developed silver grain densities are much more pronounced in irradiated than sham-irradiated samples. Closer examination reveals considerable hypothalamic, hippocampal and paraventricular labeling accentuation. Cortical and thalamic structures exhibit sparse amounts of label. Although a considerable amount of variability is encountered in the labeling patterns, additional slides are being developed to examine variations in treatment-test-pulse interactions. We believe that this approach is especially promising since some of our preliminary work suggests that we can use ³⁵S-methionine to differentiate between labeling associated with uptake and labeling associated with enhanced neural activity.

Notably, the autoradiographs do not exhibit any degree of labeling lateralization. Such localized deposition of label might have been expected using our horn exposure system, according to at least some views of microwave effects.

Conclusions

At least one mechanism has been demonstrated as a physiological correlate of enhanced behavioral proficiency in irradiated mice. It is not clear whether the changes in amino acid metabolism relate to permeability or neural activity changes, or both. Also, it is not clear that the observed effects are acute or chronic. Only acute exposure and labeling paradigms have been used. Nevertheless, it should be possible to determine whether these physiological alterations are requisite to the occurrence of behavioral changes.

Future comparisons with labeling changes induced in our random incidence exposure device might help us determine whether field orientation is crucial to the effects we have observed.

Discussion

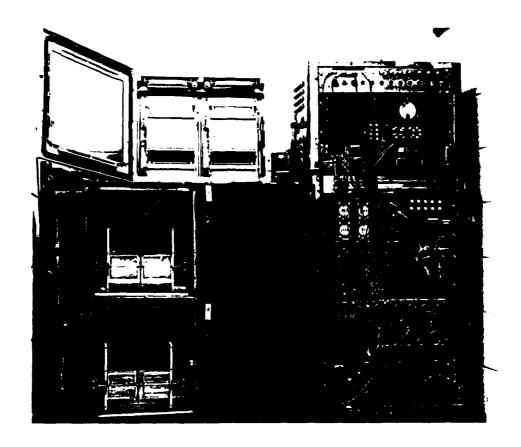
The foregoing studies indicate that microwave exposure produces reliable behavioral effects related to learning and memory. The effects are produced with two very different forms of superimposed microwave fields. Even with highly organized orthogonal there was no evidence of physiological lateralization in brain permeability or polypeptide synthesis activity. Such observations are most consistent with thermal effects induced by microwave irradiation. However, additional studies are required to validate such a hypothesis. As noted earlier, longer duration exposures and exposures administered at other times relative to the training period should enhance our understanding of the microwave effects.

To date, all of our studies have dealt with short duration, acute exposures to relatively high levels of microwave irradiation. Although these observations have begun to clarify a circumscribed set of bioeffects hypotheses, other questions are not addressed. In particular, effects upon young organisms may appear in such organisms as adults. Or, chronic exposure may be necessary to evoke a different class of consequences than those arising from acute exposure. The experimental paradigm used in our studies over the last year lends itself to direct comparisons of chronic effects with acute effects.

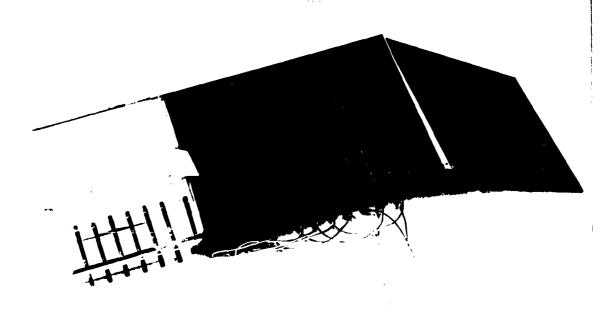
Our present observations have been limited to pulsed microwave exposure. A direct extrapolation to continuous wave exposure is not justified empirically

or theoretically. Nevertheless, continuous wave exposure can be assessed in the same paradigm as used for pulsed exposures. It should be easy to compare the results quite directly. Such comparisons have considerable potential impact for microwave bioeffects hypotheses. Average power levels can be held constant, for example, but it is clear that pulsed exposures consist of discontinuous peaks of irradiation which are often ten-fold higher than such average levels. If the bioeffects of continuous and pulsed microwave match in accord with average power as opposed to peak power levels, certain hypotheses may require restructuring.

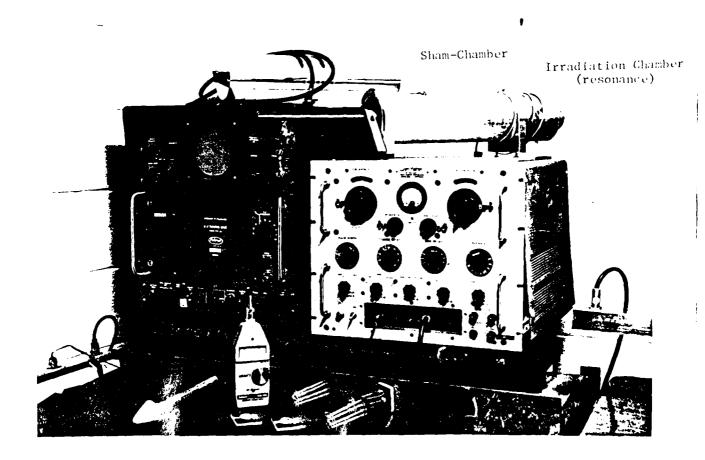
Overall, therefore, our present findings and evolved test paradigms may be used for systematic evaluations of numerous popular ideas regarding microwave effects.



Wheel-Turn Apparatus: Active Avoidance Apparatus



Jarvik Box: Passive Avoidance Apparatus



MICROWAVE IRRADIATION EQUIPMENT: Generator, Analysor, Power Meter, Resonant Chamber and Mouse Restraining Cayes

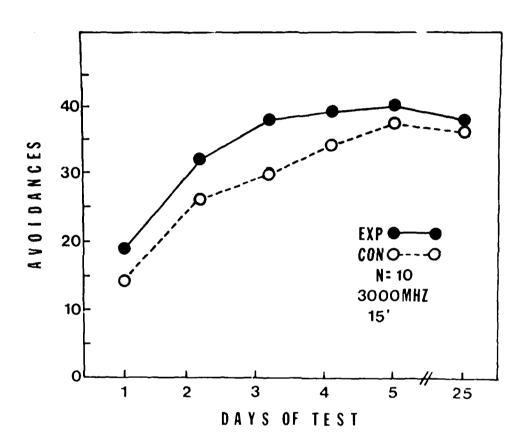


Figure 1: Sample avoidance data from one repetition of active avoidance studies

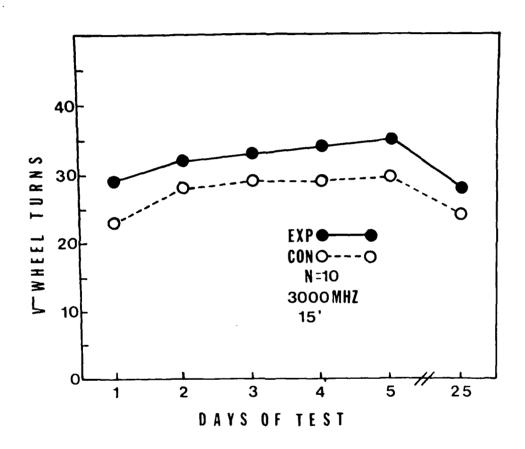


Figure 2: Wheel turn activity associated with active avoidance training

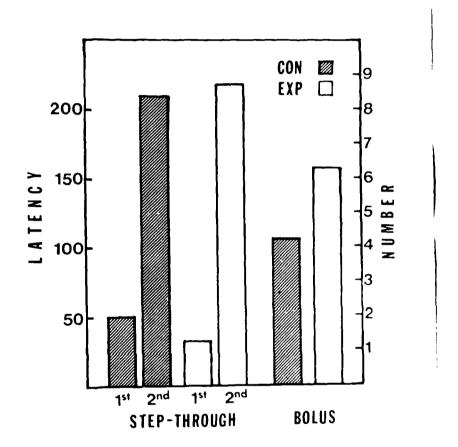


Figure 3: Summary of results from passive avoidance tests using the Jarvik box

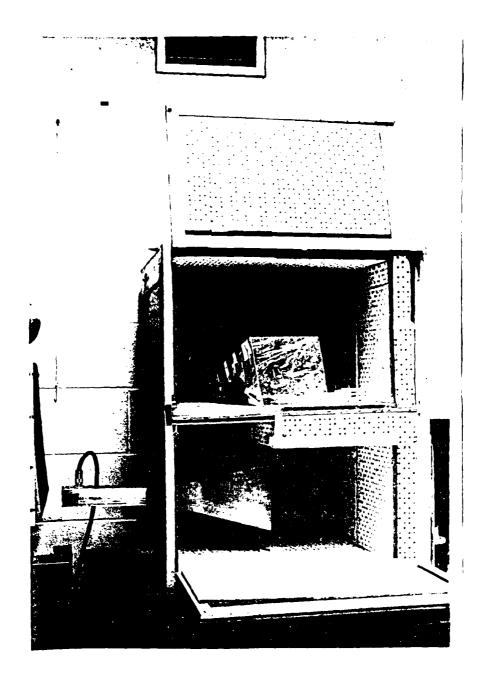


Figure 4: Organized field, irradiation and sham-irradiation chambers

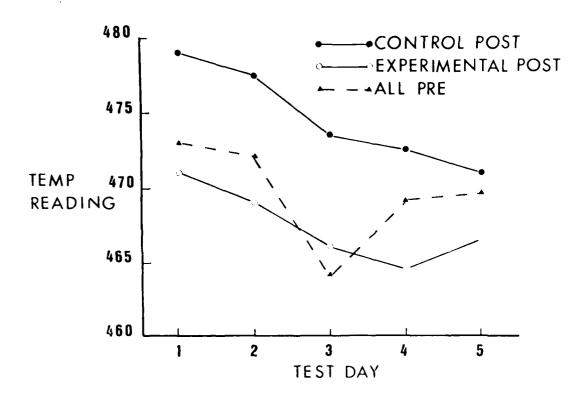


Figure 5: Summary of thermister readings after active avoidance training.

Pre-irradiation or sham-irradiation groups did not differ

significantly and were combined. Larger numbers associated

with lower temperatures.

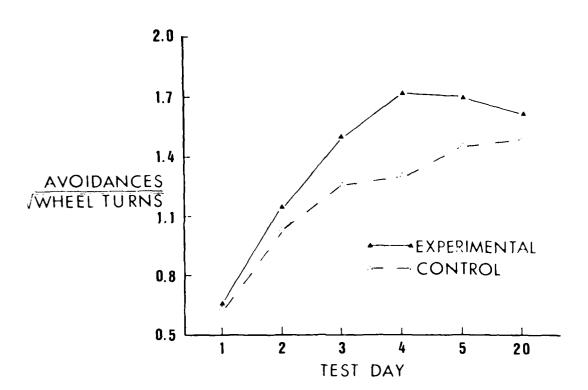


Figure 6: Response effectiveness summarized in active avoidance study

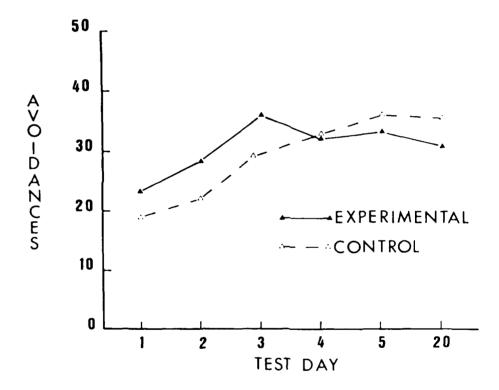


Figure 7: Summary of avoidance performance in active avoidance training.

Exposure duration = 30 mins.

